89 Rec'd PCT/PTO 02 SEP 1997

FORM PTO-1390 (REV 10-95) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: SCH 1526

U.S. APPLICATION NO (If known, see 37 CFR 1.5): 08/894767

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP96/00823	29 February 1996	01 March 1995

TITLE OF INVENTION: PROCESS AND COMPOUNDS FOR DETECTION OF ANALYTES USING REMANENCE MEASUREMENT, AND USE THEREOF

APPLICANT(S) FOR DO/EO/US: WEITSHCIES, Werner et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [XX] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendemnts has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [XX] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [XX] Other items or information: Letter re: Article 34 amendments

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/EP96/00823	SCH 1526

	CALCULATIONS	PTO USE ONLY
17 [XX] The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Search Report has been prepared by the EPO or JPO .... $ 910.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) .... $680.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... $750.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... $1010.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $94.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 910.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	38 - 20 =	18	X $22.00	$ 396.00	
Independent claims	5 - 3 =	2	X $ 80.00	$ 160.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 260.00	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 1,596.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 0	
SUBTOTAL =				$ 1,596.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 1,596.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 1,596.00	
				Amount to be: refunded	$
				charged	$

a. [XX] A check in the amount of $ 1,596.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. ______________ in the amount of $______________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3402. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
LAW OFFICES
MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
ARLINGTON COURTHOUSE PLAZA I
SUITE 1400
2200 CLARENDON BOULEVARD
ARLINGTON, VIRGINIA 22201
703-243-6333

SIGNATURE

Anthony J. Zelano
NAME

27,969
REGISTRATION NUMBER

Filed: 02 September 1997 (Monday was a Holiday)
AJZ/lyw

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE

International Application No. : PCT/EP96/00823

International Filing Date : 29 February 1996

Priority Date Claimed : 01 March 1995

Applicants (DO/EO/US) : WEITSCHIES, Werner et al.

Title: PROCESS AND COMPOUNDS FOR DETECTION OF ANALYTES USING REMANENCE MEASUREMENT, AND USE THEREOF

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

SIR:

Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend this application as follows:

IN THE CLAIMS:

Claim 4, line 1: Change "claims 2 to 3," to -- claim 2, --.

Claim 5, line 1: Change "claims 1 to 4," to -- claim 1, --.

Claim 6, line 1: Change "claims 4 and 5," to -- claim 3, --.

Claim 7, line 1: Change "claims 3 and 4," to -- claim 3, --.

Claim 8, line 1: Change "claims 1 to 7," to -- claim 1, --.

Claim 9, line 1: Change "claims 1 to 8," to -- claim 1, --.

Claim 10, line 1: Change "claims 1 to 8," to -- claim 1, --.

Claim 11, line 1: Change "claims 1 to 10," to -- claim 1, --.

Claim 13, line 1: Change "claims 1 to 12," to -- claim 1 --.

Claim 16, line 1: Change "claims 1 and 15," to -- claim 1, --.

Claim 17, line 1: Change "claims 1 and 16," to -- claim 1, --.

Claim 18, line 1: Change "claims 1 and 17," to -- claim 1, --.

Claim 19, lines 1 and 2: Change "claims 1 and 18," to -- claim 1, --.

Claim 20, lines 1 and 2: Change "claims 1-18" to -- claim 1 --.

Claim 21, lines 1 and 2: Change "claims 1-18" to -- claim 1 --

Claim 23, lines 1 and 2: Change "claims 1-18" to -- claim 1 --,

Claim 24, lines 1 and 2: Change "claims 11 and 12" to -- claim 11 --.

Claim 25, line 1: Change "claims 1 and 18," to -- claim 1, --.

Claim 32, line 1: Change "claims 26 to 31," to -- claim 26, --.

Claim 33, (Amended.) Use of [the] compounds [according to one of claims 19 to 23,] which consist of combinations of stable or quasistable ferrimagnetic or ferromagnetic substances with structure-specific substances in processes according to claim[s] 27 [to 32].

Claim 35, lines 1 and 2: Change "claims 27 to 32," to -- claim 27, --.

Claim 36, lines 1 and 2: Change "claims 26 to 32," to -- claim 26, --.

Claim 37, lines 1 and 2: Change "claims 26 to 32," to -- claim 26, --.

Claim 38, line 1: Change "claims 36 and 37" to -- claim 36, --.

REMARKS

The principal purpose of this Preliminary Amendment is to avoid the multiply dependent claim fees.

Respectfully submitted,

Anthony J. Zelano (Reg. No. 27,969)
Attorney for Applicants
MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
Arlington Courthouse Plaza I
2200 Clarendon Boulevard, Suite 1400
Arlington, Virginia 22201
Direct Dial: (703) 812-5311
Facsimile No.: (703) 243-6410
E-Mail Address: zelano@mwzb.com

Filed: **02 September 1997 (Tuesday)**
AJZ/lyw

Process and Compounds for Detection of Analytes Using Remanence Measurement, and Use Thereof

The invention relates to the object that is characterized in the claims, i.e., a process for qualitative and/or quantitative detection of analytes in liquid and solid phases using remanence measurement, compounds that are suitable for this purpose, and their use in analytic chemistry.

It is known that quantitative immunoassays, as well as other binding assays (e.g., receptor binding assays) make it possible to determine a very large number of substances that can also be of biological relevance in samples of varying compositions. Generally, however, only one parameter per sample in an assay is determined in this way. An existing survey of the various processes is: T. Chard [An Introduction to Radioimmunoassay and Related Techniques: Laboratory Techniques in Biochemistry and Molecular Biology, 4th ed., Elsevier Science Publishers, Amsterdam (1990)]. The basis of all binding assays is the high detection sensitivity of compounds that are labeled with isotopes or by some other means in combination with the high specificity of ligand-receptor reactions.

The known assay processes have the following drawbacks, however:

- The processes for simultaneous determination of various analytes within the same sample are based on the binding of varying radio-, fluorescence- or enzymological-labeled probes to the analytes. In this

In the process that is disclosed in publication JP3-220442A, the determination of an antigen titre by measuring the extent of antibody-mediated aggregation (agglutination) is performed with the aid of a process for measuring the particle sizes of agglomerated magnetic particles that is disclosed in the publication. The process for determining particle size consists in connecting a magnetic field that penetrates the stationary, deposited sample and measuring the residual magnetic flux density of the agglomerated magnetic particles.

Ferrofluids that contain superparamagnetic particles made of chromium dioxide with antibodies that are coupled thereto are known from publication US-4,661,408. It is claimed there that these particles have very short intrinsic relaxation times; they therefore do not meet the requirements for use in remanence measurements.

case, the unbound or bound activity of the probe for quantitative determination of the analyte is generally measured after subsequent separation and washing. In this case, the amounts of usable different probe labels are very limited. Thus, for example, in the case of different radioisotopes as probe labeling, so-called overlapping phenomena occur, which lead to a rapid loss of the quantitative accuracy of individual signals. Combining various enzymes as probe labels causes comparable problems, whereby implementation is additionally hampered here by the necessary search for reaction conditions that allow the simultaneous determination of enzyme reactions in a system.

- The sensitivity of the process is limited by, for example, non-specific interactions between matrix and probe, or else by limited labeling capability of the probe (low specific activity).
- The successful implementation of the process often requires that the sample material obtained be worked up (e.g., production of serum or plasma from whole blood, extraction of samples with organic solvents, concentration of the analyte using chromatographic processes, etc.).
- For successful implementation of the processes, separation and washing steps, which ensure the separation of bound and unbound receptors or ligands, are essential.

- For implementation of radioimmunoassays, the use of radiating nuclides, which are costly and complicated to handle, is necessary.
- In practice, the storage of the used markers often causes problems since they either are not stable (as in the case of radioimmunoassays) and therefore always have to be made fresh or else react to environmental influences in a sensitive manner.

The object of this invention was therefore to find a novel process and substances that are superior to the above-mentioned prior art.

This object is achieved by this invention.

It has been found that the qualitative and/or quantitative detection of analytes in liquid and/or solid phases is possible if stable or quasi-stable ferromagnetic or ferrimagnetic substances are used as magnetic labeling that is to be identified in immunoassays or binding assays and the remanent magnetization of the sample is determined as a measurement variable.

Below, processes that overcome the drawbacks of the known processes are first described.

The processes according to the invention are based on the use of colloidal ferromagnetic or ferrimagnetic substances, also referred to below as magnetic labeling, that are combined with substances that are to be identified -- also referred to below as analytes -- or structure-specific substances. Such combinations of magnetic labelings with analytes or structure-specific substances are also referred to below as magnetic markers. The

use of the term colloidal substances is intended to describe both the range of sizes of the particles or substances in the range of sizes of colloids, i.e., the range of 1 nm up to about 1000 nm, and their use as a dispersed phase in a suitable dispersion medium, which is aqueous in most cases. Colloidal substances, which are also referred to below as particles, can also be present in dried form or frozen for the purpose of improved shelf life and transportability; during the execution of measurements, however, they are generally present in the liquid-phase dispersed state.

An important principle of the invention is that the time-dependence of the magnetization of ferromagnetic or ferrimagnetic colloidal substances after an external magnetizing field is shut off depends in a very sensitive manner on the material and form (anisotropy constants of the particle material used), volume and temperature of the particles used. This is caused by rotation of the internal magnetizing vector within the particles. This mechanism is referred to as Neelian relaxation. If the magnetization of the particles relaxes within the measurement time, the superparamagnetism is referred to as intrinsic. Particles whose Neelian relaxation times are considerably longer than the observation period are referred to as remanent particles or as stable particles. Particles whose Neelian relaxation times are on the order of the observation period are referred to as quasi-stable particles.

Another essential principle of the invention is that the magnetization of a totality of freely-movable stable or quasi-

stable ferromagnetic or ferrimagnetic colloidal particles relaxes within the measurement time owing to a second mechanism after an external magnetizing field is shut off. In this case, rotation of the entire colloidal particle occurs within the surrounding liquid, whereby the time constant depends on the hydrodynamic diameter of the particles including the shell, the viscosity of the carrier liquid, and temperature. These parameters are determined mainly by the environment of the particles. The mechanism is referred to as Brownian relaxation or extrinsic superparamagnetism.

The process according to the invention for qualitative and/or quantitative detection of analytes in liquid and solid phases is carried out by

i) structure-specific substances first being labeled with ferrimagnetic or ferromagnetic substances, and then

ii) these magnetically labeled structure-specific substances being used in a sample that is to be measured,

iii) with the sample to be measured being magnetized with the aid of a magnetic field of suitable intensity that is applied from the outside and,

iv) after the external field is shut off, the remanence of the magnetization of the colloidal particles (magnetic labeling) being measured with the aid of magnetic field sensors,

whereby the remanence that occurs due to specific binding and its extent are used for analysis.

In the processes of the prior art, the discrimination that was possible only in exceptional cases between bound and unbound markers is made possible in the process according to the invention by the use of the fading remanence (i.e., extrinsic superparamagnetism) of the unbound, magnetic markers in the liquid phase. The totality of the bound magnetic markers, however, show a measurable remanence that depends on the particle material. In measuring the remanence of the sample to be determined, only the portion of bound magnetic markers is thus detected. The process is therefore also referred to below as measuring binding remanence or binding remanence measurement and is based on the quantitative detection of bound structure-specific markers.

In other words, the determination of the analyte can also be done without expensive separation and washing steps since only the stable or quasi-stable ferromagnetic or ferrimagnetic substances that are specifically bound to the analytes (which are combined with structure-specific substances for the purpose of imparting a specificity) cause remanence, while the magnetization of excess, unbound stable or quasi-stable ferromagnetic or ferrimagnetic substances that are simultaneously present in the sample (which are also combined with structure-specific substances) fades even before the beginning of the measurement by extrinsic superparamagnetism.

In another embodiment of the invention, the process is modified in such a way that instead of the structure-specific substances, the analytes themselves are combined with the stable

or quasi-stable ferromagnetic or ferrimagnetic substances, as is often used analogously in, e.g, the execution of competitive radioimmunoassays. The structure-specific substance then has to be additionally added to the samples.

The process can also be implemented as a multianalyte assay, however, which makes possible direct simultaneous determination of several different analytes in liquids or solids. To this end, it is necessary first to label the analytes with different ferromagnetic or ferrimagnetic colloidal substances with sufficiently discrete coercive field intensities. During the binding of the magnetic markers, a magnetizing field (primary magnetizing field) is applied, which causes all magnetic markers that are contained in the sample to be oriented along their simple axis.

Then, the remanence of the sample is determined. In further steps, the sample is demagnetized with external counter-fields (running counter to the primary magnetizing field), which are matched in their intensity to the coercive field intensities of the magnetic labelings. As a result, it is possible always to re-orient specifically only those labelings whose coercive field intensities are lower than the applied magnetizing field. The remanence of the sample is again determined in each case between the individual steps of the demagnetization.

The portion of different bound magnetic markers can thus be quantified based on the measured remanences of the sample in each individual demagnetization step.

This process makes it possible to determine several analytes per sample simultaneously.

The process according to the invention, as well as variants of the process, can be used in fertility, histocompatibility, allergology, infectiology, hygiene, genetics, virology, bacteriology, toxicology, pathology, environmental analysis, and medical diagnosis.

The detection of binding remanence is carried out with known measurement arrangements that are suitable for this purpose, such as, for example, Superconducting Quantum Interference Devices (SQUIDs), whereby first magnetization is done with the aid of a suitable magnetic field, and then the measurement of the remanence of the magnetized structure-specific substance or signals that are dependent thereon is done using sensitive magnetic field sensors after the field is shut off.

During measurement, the sample can advantageously be moved. Especially advantageous is modulation of the signal by vibration or rotation of the sample. This ensures transformation of the dc-measuring signal to a higher frequency range.

For measurement -- in addition to the above-mentioned SQUIDs -- induction coils that are hooked up as gradiometers, fluxgate-magnetometers, giant magnetoresistance sensors, or magnetoresistive converters can also be used.

When sensors that can measure dc fields (e.g., SQUIDs, fluxgate-magnetometers, giant magnetoresistance sensors, or magnetoresistive converters) are used, measurement of remanence

after preceding magnetization of the sample is also possible, without the latter being moved.

A suitable measurement arrangement is depicted by way of example in Figure 1. Such an arrangement was also used in the examples below.

Another aspect of the invention relates to compounds for measuring binding remanence. Suitable compounds consist of colloidal suspensions, ferrimagnetic or ferromagnetic substances, and structure-specific substances or analytes.

Within the framework of this invention, structure-specific substances are defined as all substances that bind specifically to the desired structure, such as, e.g., antibodies, antibody fragments, biotin, or substances that bind specifically to biotin such as avidin or streptavidin, agonists that bind specifically to receptors, such as cytokines, lymphokines, endothelins or their antagonists, specific peptides and proteins, receptors, enzymes, enzyme substrates, nucleotides, ribonucleic acids, deoxyribonucleic acids, carbohydrates, lipoproteins, etc. Among them, substances are preferred whose binding constant is in the range of 10^5-10^{15} $(mol/l)^{-1}$, and especially substances whose binding constant is in the range of 10^7-10^{15} $(mol/l)^{-1}$.

As ferromagnetic or ferrimagnetic colloidal substances, all ferromagnetic and ferrimagnetic substances can be used whose intrinsic Neelian relaxation time is greater than or equal to the measuring time and which thus are stable or quasi-stable.

Especially suitable are all ferromagnetic and ferrimagnetic substances whose relaxation time is longer than 10^{-4} second at

20°C. Especially suitable are all ferromagnetic and ferrimagnetic substances whose relaxation time is longer than 1 second.

The ferromagnetic and ferrimagnetic substances can advantageously be stabilized with a shell made of oligomeric or polymeric carbohydrates, proteins, peptides, nucleotides, surfactants, synthetic polymers, and/or lipids.

The particle sizes of the ferromagnetic and ferrimagnetic substances are advantageously between 1 nm and 1000 nm. Especially preferred are particle sizes of between 2 nm and 500 nm. (The values with respect to particles sizes relate to hydrodynamic diameter.)

As ferromagnetic or ferrimagnetic substances, especially stable or quasi-stable colloidal particles that consist of iron oxides (Fe_3O_4, γ-Fe_2O_3), barium ferrite, strontium ferrite, pure iron, chromium dioxide, nickel, cobalt as well as iron oxides with manganese, copper, nickel or cobalt additives (as described in, e.g., DE 30 27 012 and DE 41 16 093), are suitable.

The production of the compounds that can be used according to the invention that consist of stable or quasi-stable ferromagnetic or ferrimagnetic substances, which are connected to structure-specific substances or analytes, is carried out by means of processes that are familiar in the area of immunochemistry, peptide chemistry, and protein chemistry. In this case, the structure-specific substance or the analyte is linked via covalent bonds to the substances that form the stabilizing shell of the ferromagnetic or ferrimagnetic

substances. As stabilizing shells, e.g., carbohydrates, peptides, nucleotides, proteins, lipids, surfactants, or polymers are suitable. Especially suitable linkage methods are, e.g., activation and coupling using carbodiimides [Jakoby and Wilchek, eds., (1974) Methods Enzymol 34], the formation of Schiff bases after periodates act on compounds that contain carbohydrates (Wichek and Bayer, eds., Methods Enzym 184:177), which optionally are then again reduced for further stabilization, coupling using glutaric dialdehyde [Heitzmann and Richards, Proc. Natl. Acad. Sci. USA, 71 (1974) 3537], the crosslinking of bromoacetylated particles with thiolylated substances [Angerer et al., Cell 9 (1976) 81], and reductive alkylation [Bayer et al., J. Histochem. Cytochem. 24 (1976) 933].

Ferromagnetic or ferrimagnetic colloidal particles can also be produced with a stabilizing shell made of the structure-specific substance or the analyte itself, by the particles being brought after production either directly into a solution of the structure-specific substance or the analyte, optionally in the presence of other adjuvants, such as, e.g., proteins, carbohydrates as well as natural, synthetic or partially synthetic surfactants, or being produced directly in the presence of structure-specific substances or analytes.

For the purpose of performing multianalyte assays, mixtures of compounds that consist of several different magnetic markers can also be used, whereby the different magnetic markers consist of combinations of different structure-specific substances or analytes that are to be identified with different ferromagnetic

or ferrimagnetic substances. The principle of this use of combinations of different magnetic markers according to the invention is that ferromagnetic or ferrimagnetic substances with varying coercive field intensities (H_c) are used as magnetic labeling for the respective structure-specific substances or analytes that are to be identified, whereby the parameters of coercive field intensity (H_c) and remanent magnetization (M_R) for the different magnetic labelings can be determined separately in advance in a known way and thus are known.

The structure-specific substances that are labeled with the ferromagnetic or ferrimagnetic colloidal substances can also be present in dried form (e.g., as lyophilizates), optionally in combination with other adjuvants which facilitate drying or increase the stability of the dried product (e.g., as lyophilizates). The production of the agents that are ready-for-use from such lyophilizates is then carried out by resuspension in a suitable suspension medium immediately before use.

Another aspect of the invention thus relates to ferromagnetic or ferrimagnetic colloidal substances that contain agents for binding remanence measurement in a suitable suspension medium.

As suspension media, all liquids are suitable in which the colloidal particles can move freely. If the measurements are carried out without separation or washing steps, the viscosity of the suspension medium that is used has to be matched to the Neelian relaxation time of the ferromagnetic and ferrimagnetic substances and the measuring time since the suspension medium

basically determines the time constant of the Brownian relaxation. Otherwise, e.g., when using particles with a short relaxation time (e.g., 0.01 second) in highly viscous suspension media (e.g., 80% glycerol) and at short measurement times (e.g., 0.0001 second after the external field is shut off), Brownian relaxation (extrinsic superparamagnetism) can be slowed to such an extent that a distinction can no longer be made between bound and unbound structure-specific markers. It is generally advantageous to use suspension media of a viscosity of less than 100 mPas.

As suspension media, water, aqueous solutions of surface-active adjuvants, such as, e.g., surfactants or oligomeric or polymeric carbohydrates and proteins, as well as mixtures of water with alcohols such as, e.g., glycerol and polyethylene glycol, are suitable, whereby water that is suitable for injection purposes is preferred. In addition, the suspension media can contain adjuvants that change the osmotic pressure, such as, e.g., common salt. In addition, buffer substances that determine pH, such as, e.g., phosphates, may be contained.

Another object of the invention is the use of compounds in the process according to the invention for measuring binding remanence.

Due to the binding identification based on physical mechanisms, non-specific measurement signals (matrix phenomena) can be largely ruled out. The specificity of the process thus depends only on the "true" specificity of the structure-specific

substance (cross reactivity of antibodies, non-specific binding of ligands).

Due to the high sensitivity of the process according to the invention, it is easy to remain under the detection limits of binding assays that are otherwise commonly encountered.

In contrast to known methods (JP-235774 and WO 91/15243), in the process according to the invention, it is not static magnetization that is measured in the presence of an external magnetizing field but rather, in the absence of a magnetizing field, the binding remanence or signals dependent thereon. Only in this way are data on the binding state of the markers made available.

In addition, this keeps the measuring signal from being influenced by diamagnetic or paramagnetic components or contaminants; this helps to further increase measuring sensitivity.

The processes for binding remanence measurement according to the invention can additionally be used to identify in vivo the retention sites of ferromagnetic or ferrimagnetic substances. In this case, it is of special advantage that ferromagnetic or ferrimagnetic substances can be used to determine binding remanence and thus the especially critical use of radioactive isotopes in humans is avoided, whereby the sensitivity of the process according to the invention reaches that of commonly used nuclear-medicine processes of gamma scintigraphy or positron emission tomography. Moreover, prior separation of unbound markers that circulate in the blood is not necessary since the

latter (in contrast to radiodiagnostic methods) does not cause any "interfering signal," and thus the detection of specifically bound markers is not affected.

According to the invention, a suspension of stable or quasi-stable ferromagnetic or ferrimagnetic substances is administered to the patient. Local application, peroral administration, and all forms of parenteral administration are suitable as administration methods. Especially suitable are intravascular forms of administration.

Colloidal substances are dispersed in the organism, whereby the distribution pattern in the body is influenced by the method of administration, as well as by various other pharmacokinetic parameters. The site-resolved determination of binding remanence can be used at different times after administration of stable or quasi-stable ferromagnetic or ferrimagnetic substances to determine pathologic states in the body, which are characterized both by unusual concentrations or by the failure of concentrations to occur.

For visualization of pathological structures in the human body, the use of stable or quasi-stable ferromagnetic or ferrimagnetic substances (magnetic markers) combined with structure-specific substances can be especially advantageous. Here, the specific binding of the magnetic markers to the pathological structure results in a specific signal, which can be determined **in vivo** according to the invention with the processes of binding remanence that are described for carrying out binding assays.

As an example of an application of stable or quasi-stable ferromagnetic or ferrimagnetic substances in combination with the process of binding remanence measurement according to the invention, the detection of tumors in the body using tumor-specific magnetic markers can be mentioned. Tumor-specific markers can be, e.g., combinations of stable or quasi-stable ferromagnetic or ferrimagnetic substances with tumor-specific substances, such as, e.g., antibodies, antibody fragments, peptides, receptors, proteins, nucleic acids, oligonucleotides, and monomeric, oligomeric, or polymeric carbohydrates.

Other examples of possible applications are the visualization of clots, arteriosclerotic plaques, inflammatory reactions, and rheumatic or rheumatoid alterations, whereby in each case it is advantageous to use as magnetic markers combinations of stable or quasi-stable ferromagnetic or ferrimagnetic substances according to the invention with specific substances for the respective pathologic structure.

The **in-vivo** measurement of the spatial distribution of stable or quasi-stable magnetic markers that are administered to a human is carried out according to the invention according to two variants:

1. Generating as homogeneous a magnetic field as possible in the advantageous body regions, shutting off the field, and measuring the spatial distribution of the binding remanence using a SQUID-multichannel sensor, as is used for, e.g., biomagnetic tests [cf. D. Drung, IEEE Trans. Appl. Supercond., 5 (1995) 2112-2117].

This sensor should enclose the measuring object as completely as possible. To produce sufficient measuring information, re-measurement with sequential rastering of the measuring object is advantageous.

2. Sequentially generating a spatially limited local field, shutting off the field, and measuring the spatial binding remanence using a single-channel sensor. The use of a multichannel sensor is also possible.

With both methods, both magnetization of the measurement object and measurement of the resulting magnetic field in all three spatial directions is to be preferred to ensure that maximum information is collected.

The evaluation of the measuring data can be done using a suitable approximation process. Thus, e.g., the model of the magnetic dipole, multipole, or multi-dipole can be taken as the point of departure. The special parameters of the model, particularly the locations of the dipoles or multipoles, are then found using the approximation process, which minimizes the deviations between measurement data and model parameters. The spatial distribution of the magnetized particles can be determined from these parameters in a way known in the art.

An analogous approach is known and has proven itself in analyzing the magnetic fields of bioelectric currents.

For the measurement of binding remanence **in vivo**, basically the same substances -- or agents that are prepared from them -- as are also used in **in vitro** tests are suitable.

Especially suitable for **in-vivo** application are magnetic labelings that are biodegradable and compatible. This is especially true of magnetic labelings that consist of iron oxides or combinations of iron oxides with manganese or cobalt. Magnetic particles to which structure-specific substances can be linked according to the known processes are used in, e.g., nuclear spin tomography and are described in, i.a., WO 92/12735, WO 92/22586 as well as EP 0 186 616. Another aspect of the invention thus relates to the use of magnetic labelings in an **in-vivo** process for measuring binding remanence.

In connection with the **in-vivo** application of binding remanence measurement, structure-specific substances are defined especially as all substances that bind specifically to structures of the human body that are to be identified. Especially suitable are antibodies, antibody fragments, agonists that bind specifically to receptors or their antagonists, specific peptides and proteins, receptors, enzymes, enzyme substrates, nucleotides, ribonucleic acids, deoxyribonucleic acids, carbohydrates, or lipoproteins. Of the agonists that bind to receptors, especially cytokines, lymphokines, or endothelins are suitable.

Well suited are all structure-specific substances that have a binding constant in the range of 10^5-10^{15} $(mol/l)^{-1}$. Especially suitable are all structure-specific substances that have a binding constant in the range of 10^7-10^{15} $(mol/l)^{-1}$.

The following examples are used to provide a more detailed explanation of the object of the invention, without intending that it be limited to these examples.

Example 1

100 μg of a monoclonal antibody to collagen III, referred to below as anticollagen III, is dissolved in 500 μl of a 0.1 M sodium bicarbonate solution. 1 ml of dextran-coated magnetite suspension (Meito Sangyo) with 1 mol of Fe/l and a particle size of about 40 nm (hydrodynamic diameter) is buffered with 0.1 M sodium bicarbonate on a Sephadex column (Pharmacia PD 10). 0.5 ml of 10 mmol sodium periodate solution is added to the suspension. The solution is allowed to stand in the dark for 2 hours. Then, it is eluted on a PD 10 with 0.1 M sodium bicarbonate solution. The anticollagen III solution is added to the suspension. The mixture is allowed to stand in the dark for 3 hours at 4°C. Then, 5 mg of $NaBH_4$ is added as a solid and briefly swirled. The mixture is allowed to stand in the dark for 8 hours at 4°C. Then, the magnetite-labeled anticollagen III (referred to below as mag-anticollagen III) is eluted via a PD 10 column with phosphate-buffered common salt solution (PBS, pH 7.4).

A solution of 5 μg of collagen III in 200 μl of buffer [phosphate-buffered common salt solution (PBS)] is incubated in a polystyrene sampling vessel. (The sampling vessel serves in this case as a solid phase, to which a portion of the collagen is attached.) The liquid phase is then discarded. The sampling vessel is flushed three times with phosphate-buffered common salt solution, containing 0.1% Tween(R) 20 (referred to as PBST below), to wash out unattached collagen. In the sampling vessel, 5 μl of mag-anticollagen III, dissolved in 200 μl of PBST, is then added.

It is incubated for 1 hour at room temperature. Then, the sample is magnetized in a magnetically shielded chamber in a field with an intensity of 2 mT 4 cm below the squid detector (see Figure 1). After the magnetic field is shut off, the sample is measured. To this end, the sample is removed from the magnetizing coil during measurement. The remanence is produced from the difference of the determined magnetic flux densities -- after the magnetic field is shut off -- before and after the sample is removed from the measuring field. In the case of this example, a remanence was found.

Example 2

A solution of 5 μg of collagen V in 200 μl of PBS buffer of pH 7.4 is incubated in a sampling vessel made of polystyrene. Then, the liquid phase is discarded. The sampling vessel is flushed three times with PBST washing buffer of pH 7.4. 5 μl of mag-anticollagen III, produced according to Example 1, in 200 μl of PBST is added to the sample. It is incubated for 1 hour at room temperature. Then, the sample is magnetized in a magnetically shielded chamber in a field with an intensity of 2 mT 4 cm below the SQUID detector (see Figure 1). After the magnetizing field is shut off, the sample is measured. To this end, the sample is removed from the magnetizing coils during measurement. In the sample that contains collagen V, no remanence can be detected.

Example 3

From 10 ml of a 1.9 mg/ml collagen III solution in PBS (pH 7.4), 5 ml each of the following dilutions is produced:

1,000 ng/ml, 100 ng/ml, 10 ng/ml

In each case 1 ml apiece is pipetted from each dilution into a polystyrene tube (2.5 ml capacity). The three sample tubes are inhibited for 1 hour at 37°C. Then, the contents of the tubes are discarded. The tubes are washed three times with 1 ml of PBST each.

1 ml of a 1:100 dilution of the magnetite-labeled antibody, produced according to Example 1, is added to each tube. The tubes are allowed to stand for 1 hour at room temperature. Then, the samples are magnetized (2 mT) with the measuring arrangement outlined in Figure 1 and, after the magnetizing field is shut off, the samples are measured. To this end, the samples are removed from the magnetizing coils during measurement. The evaluation of the measured signals is a measure of binding remanence and yields the relationship shown in Figure 2.

Claims

1. Process for qualitative and/or quantitative detection of analytes in liquid and/or solid phases, characterized in that stable or quasi-stable ferromagnetic or ferrimagnetic substances are used as magnetic labeling that is to be identified in immunoassays or other binding assays and the remanent magnetization of the sample is determined as a measurement variable.

2. Process for qualitative and/or quantitative detection of analytes in immunoassays or other binding assays, wherein, at the time of measurement, bound magnetic markers in their totality produce a remanent magnetization of the sample, while at the time of measurement the magnetization of unbound magnetic markers that are present in the sample in their totality fades owing to extrinsic superparamagnetism.

3. Process for qualitative and/or quantitative detection of analytes in liquid and solid phases, wherein

(i) first structure-specific substances are labeled with ferrimagnetic or ferromagnetic substances, and then

(ii) these magnetic labeled structure-specific substances are used in a sample that is to be measured,

(iii) the sample to be measured is magnetized with the aid of a magnetic field of suitable intensity that is applied from the outside and,

(iv) after the external field is shut off, the remanence of the magnetization of the colloidal particles is measured with the aid of magnetic field sensors,

whereby the remanence that occurs due to specific binding and its extent are used for analysis.

4. Process according to claims 2 and 3, wherein instead of the structure-specific substances, analytes that are to be identified are labeled with ferrimagnetic or ferromagnetic substances and the structure-specific substances are added to the samples that are to be measured.

5. Process according to claims 1 to 4, wherein the structure-specific substances are antibodies, antibody fragments, biotin, or substances that bind specifically to biotin such as avidin or streptavidin, agonists that bind specifically to receptors or their antagonists, specific peptides and proteins, receptors, enzymes, enzyme substrates, nucleotides, ribonucleic acids, deoxyribonucleic acids, carbohydrates, or lipoproteins.

6. Process according to claims 4 and 5, wherein the structure-specific substances have a binding constant in the range of 10^5-10^{15} $(mol/l)^{-1}$.

7. Process according to claims 3 and 4, wherein the structure-specific substances have a binding constant in the range of 10^7-10^{15} $(mol/l)^{-1}$.

8. Process according to claims 1 to 7, wherein the sample is moved during the measurement and thus the sample signal is modulated.

9. Process according to claims 1 to 8, wherein induction coils that are hooked up as gradiometers, fluxgate-magnetometers, giant magnetoresistance sensors, or magnetoresistive converters are used as magnetic field sensors.

10. Process according to claims 1 to 8, wherein SQUIDs are used as magnetic field sensors.

11. Process according to claims 1 to 10, wherein simultaneous determination of several different analytes in liquids or solid substances is carried out by step-by-step magnetization of the sample to be measured.

12. Process according to claim 11, wherein for simultaneous quantitative determination of analytes, different ferromagnetic or ferrimagnetic substances with discrete coercive field intensities are used.

13. Process according to claims 1 to 12, wherein the intrinsic Neelian relaxation times of the ferromagnetic and ferrimagnetic substances that are used are greater than the measuring time.

14. Process according to claim 13, wherein the Neelian relaxation times of the ferromagnetic and ferrimagnetic substances that are used are longer than 10^{-4} seconds at 20°C.

15. Process according to claim 13, wherein the Neelian relaxation times of the ferromagnetic and ferrimagnetic substances that are used are longer than 1 second at 20°C.

16. Process according to claims 1 to 15, wherein the ferromagnetic and ferrimagnetic substances have a particle size in the range of 1 to 1000 nm.

17. Process according to claims 1 to 16, wherein the ferromagnetic and ferrimagnetic substances have a particle size in the range of 2 to 500 nm.

18. Process according to claims 1 to 17, wherein the ferromagnetic and ferrimagnetic substances are stabilized with a shell that is made of oligomeric or polymeric carbohydrates, proteins, peptides, nucleotides, surfactants, synthetic polymers, and/or lipids.

19. Compounds for use in processes according to claims 1 to 18, wherein they consist of combinations of stable or quasi-stable ferrimagnetic or ferromagnetic substances with structure-specific substances.

20. Compounds for use in the process according to claims 1-18, wherein the ferrimagnetic or ferromagnetic particles have a Neelian relaxation time that is longer than 10^{-4} second.

21. Compounds for use in the process according to claims 1-18, wherein the ferrimagnetic and ferromagnetic particles have a Neelian relaxation time that is longer than 1 second.

22. Compound according to claim 19, wherein the structure-specific substances are antibodies, antibody fragments, agonists that bind specifically to receptors, cytokines, lymphokines, endothelins or their antagonists, other specific peptides and proteins, receptors, enzymes, enzyme substrates, nucleotides, ribonucleic acids, deoxyribonucleic acids, carbohydrates, or lipoproteins.

23. Compound for use in the process according to claims 1-18, wherein the ferromagnetic or ferrimagnetic substances are stable or quasi-stable colloidal particles that are made of iron oxides, barium ferrite, strontium ferrite, pure iron, chromium

dioxide, nickel, and cobalt, as well as iron oxides with manganese, copper, nickel, or cobalt additives.

24. Agents for use in the process according to claims 11 and 12, wherein they contain several ferromagnetic or ferrimagnetic substances with various coercive field intensities.

25. Use of the processes according to claims 1-18 in fertility, histocompatibility, allergology, infectiology, hygiene, genetics, virology, bacteriology, toxicology, pathology, environmental analysis, or medical diagnosis.

26. Process for the detection of ferromagnetic or ferrimagnetic substances that are introduced into the human body or that are applied on the human body, wherein the remanence of the magnetization of the ferromagnetic or ferrimagnetic substances is determined after a magnetizing field is shut off.

27. Process for the detection of ferromagnetic or ferrimagnetic substances that are introduced into the human body or applied on the human body, characterized in that first

(i) structure-specific substances are labeled with ferrimagnetic or ferromagnetic substances, and then

(ii) these magnetic labeled structure-specific substances are introduced into the living organism or applied to the organism,

(iii) an advantageous volume of the organism is magnetized with the aid of a magnetic field that is applied from the outside and,

(iv) after the external field is shut off, the remanence of the magnetization of the magnetic markers is measured with the aid of magnetic field sensors.

28. Process according to claim 27, wherein antibodies, antibody fragments, agonists that bind specifically to receptors or their antagonists, specific peptides and proteins, receptors, enzymes, enzyme substrates, nucleotides, ribonucleic acids, deoxyribonucleic acids, carbohydrates, or lipoproteins are used as structure-specific substances.

29. Process according to claim 28, wherein the agonists or antagonists that bind specifically to receptors are cytokines, lymphokines, endothelins, or their antagonists.

30. Process according to claim 28, wherein the structure-specific substances have a binding constant in the range of 10^5–10^{15} $(mol/l)^{-1}$.

31. Process according to claim 28, wherein the structure-specific substances have a binding constant in the range of 10^7–10^{15} $(mol/l)^{-1}$.

32. Process according to claims 26 to 31, wherein Superconducting Quantum Interference Devices (SQUIDs), induction coils, fluxgate-magnetometers, giant magnetoresistance sensors, or magnetoresistive converters are used as magnetic field sensors.

33. Use of the compounds according to one of claims 19 to 23, in processes according to claims 27 to 32.

34. Use of ferrimagnetic or ferromagnetic substances in the process according to claim 26.

35. Agents for use in processes according to claims 27 to 32, wherein they contain a mixture of different ferrimagnetic or ferromagnetic substances with structure-specific substances.

36. Compounds for use in processes according to claims 26 to 32, wherein the Neelian relaxation time of the ferromagnetic or ferrimagnetic substances is longer than 10^{-4} second at 37°C.

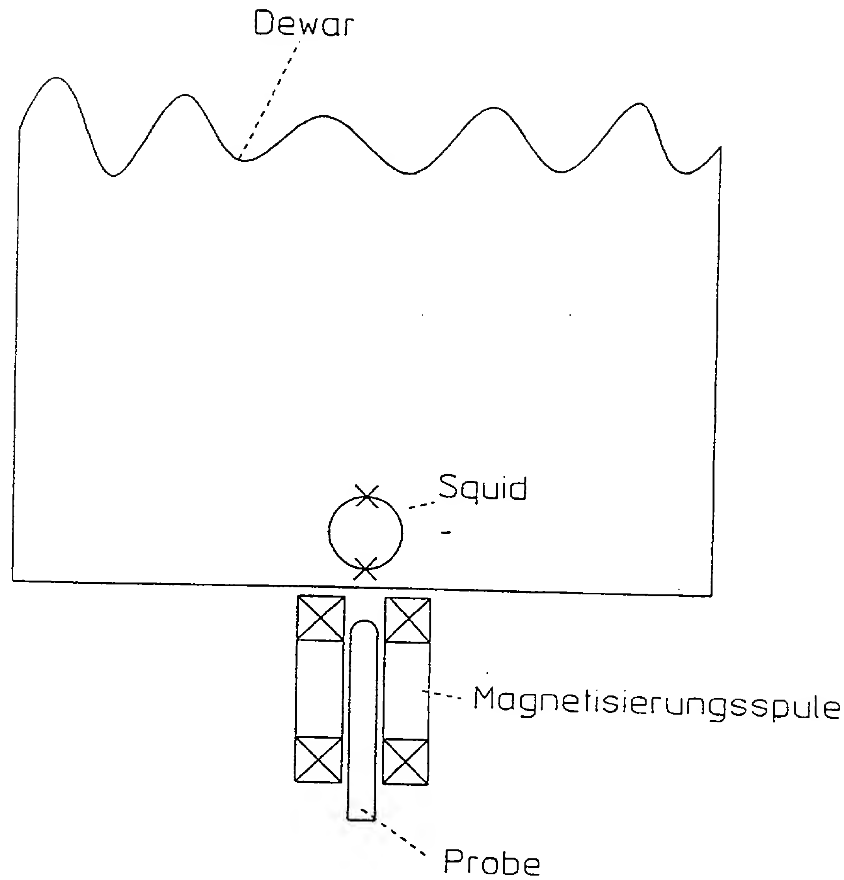
37. Compounds for use in processes according to claims 26 to 32, wherein the Neelian relaxation time of the ferromagnetic or ferrimagnetic substances is longer than 1 second at 37°C.

38. Compounds according to claims 36 and 37, wherein the ferrimagnetic or ferromagnetic substances are iron oxides or iron oxides with manganese, copper, nickel, or cobalt additives.

Abstract

The invention relates to a process for quantitative detection of analytes in liquid and solid phases with the aid of binding remanence measurement, compounds that are suitable for this purpose, and their use in analytic chemistry.

Fig. 1



[Key:]

Dewar = Dewar

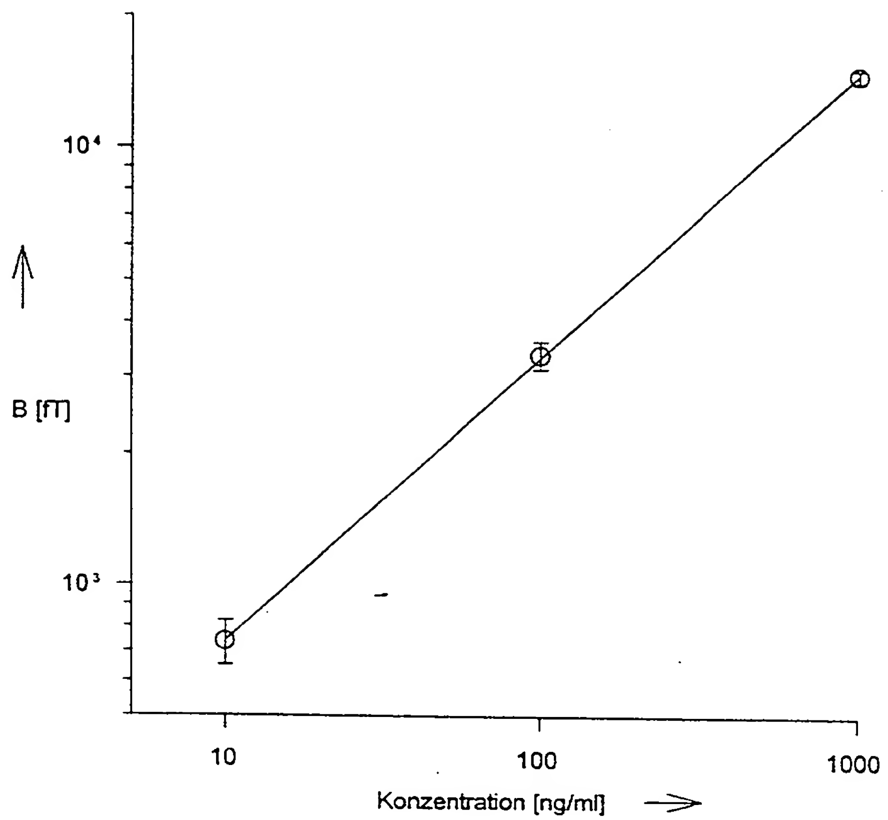
Squid = Superconducting Quantum Interference Device

Magnetisierungsspule = magnetizing coil

Probe = sample

Probe wird während der Messung entfernt = The sample is removed during measurement

Fig. 2



[Key:]

Konzentration [ng/ml] = concentration [ng/ml]

Docket No.
SCH 1526

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROCESS AND COMPOUNDS FOR DETECTION OF ANALYTES USING REMANENCE MEASUREMENT, AND USE THEREOF

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 29 February 1996 as United States Application No. or PCT International Application Number PCT/EP96/00823

and was amended on ______________________

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
195 08 772.0	Germany	01 March 1995	☒
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112. I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

14

I. William Millen (Reg. No. 19,544)
John L. White (Reg. No. 17,746)
Anthony J. Zelano (Reg. No. 27,969)
Alan E.J. Branigan (Reg. No. 20,565)
John R. Moses (Reg. No. 24,983)
Harry B. Shubin (Reg. No. 32,004)
Brion P. Heaney (Reg. No. 32,542)
Richard J. Traverso (Reg. No. 30,595)
Diana Hamlet-King (Reg. No. 33,302)
John A. Sopp (Reg. No. 33,103)
Richard E. Kurtz (Reg. No. 33,936)
Richard M. Lebovitz (Reg. No. 37,067)
John H. Thomas (Reg. No. 33,460)
Luan Cao Do (Reg. No. 38,434)

Send Correspondence to: **~~MILLEN, WHITE, ZELANO & BRANIGAN, P.C.~~**
Arlington Courthouse Plaza I
2200 Clarendon Blvd., Suite 1400
Arlington, VA 22201

Direct Telephone Calls to: *(name and telephone number)*
Anthony J. ZELANO 703-812-5310

1-0

Full name of sole or first inventor **WEITSCHIES, Werner**	
Sole or first inventor's signature	Date 31/1/98
Residence **Germany**	
Citizenship **Germany**	
Post Office Address **Gneisenaustrasse 65**	
D-10961 Berlin, GERMANY DEX	

2-0

Full name of second inventor, if any **KÖTITZ, Roman**	
Second Inventor's signature	Date
Residence **Germany**	
Citizenship **Germany**	
Post Office Address **Arnold-Zweig-Strasse 12**	
D-13189 Berlin, GERMANY DEX	

54155

3 OD

Full name of third inventor, if any **TRAHMS, Lutz**	
Third inventor's signature	Date
Residence **Germany**	
Citizenship **Germany**	
Post Office Address **Reinhardtstrasse 5**	
D-12103 Berlin, GERMANY DEX	

4 OD

Full name of fourth inventor, if any **BUNTE, Thomas**	
Fourth inventor's signature Thomas Bunte	Date 02.02.98
Residence **Germany**	
Citizenship **Germany**	
Post Office Address **Hilberstrasse 4**	
D-12307 Berlin, GERMANY DEX	

Full name of fifth inventor, if any	
Fifth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

51155

Express Mail Label No.

Docket No.
SCH 1526

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROCESS AND COMPOUNDS FOR DETECTION OF ANALYTES USING REMANENCE MEASUREMENT, AND USE THEREOF

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 29 February 1996 as United States Application No. or PCT International Application Number PCT/EP96/00823

and was amended on ______________________

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Not Claimed
195 08 772.0	Germany	01 March 1995	☒
			☐
			☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112. I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

I. William Millen (Reg. No. 19,544)
John L. White (Reg. No. 17,746)
Anthony J. Zelano (Reg. No. 27,969)
Alan E.J. Branigan (Reg. No. 20,565)
John R. Moses (Reg. No. 24,983)
Harry B. Shubin (Reg. No. 32,004)
Brion P. Heaney (Reg. No. 32,542)
Richard J. Traverso (Reg. No. 30,595)
Diana Hamlet-King (Reg. No. 33,302)
John A. Sopp (Reg. No. 33,103)
Richard E. Kurtz (Reg. No. 33,936)
Richard M. Lebovitz (Reg. No. 37,067)
John H. Thomas (Reg. No. 33,460)
Luan Cao Do (Reg. No. 38,434)

Send Correspondence to: **MILLEN, WHITE, ZELANO & BRANIGAN, P.C.**
Arlington Courthouse Plaza I
2200 Clarendon Blvd., Suite 1400
Arlington, VA 22201

Direct Telephone Calls to: *(name and telephone number)*
Anthony J. ZELANO 703-812-5310

Full name of sole or first inventor **WEITSCHIES, Werner**
Sole or first inventor's   Date
Residence **Germany**
Citizenship **Germany**
Post Office Address **Gneisenaustrasse 65**
D-10961 Berlin, GERMANY

Full name of second inventor, if any **KÖTITZ, Roman**
Second inventor's signature   Date
Residence **Germany**
Citizenship **Germany**
Post Office Address **Arnold-Zweig-Strasse 12**
D-13189 Berlin, GERMANY

Full name of third inventor, if any **TRAHMS, Lutz**
Third inventor's signature   X   Date 3.2.98
Residence **Germany**
Citizenship **Germany**
Post Office Address ~~**Reinhardtstrasse 5**~~ STEGLITZER DAMM 48
D-~~12103~~ Berlin, GERMANY

12169

Full name of fourth inventor, if any **BUNTE, Thomas**
Fourth inventor's signature   Date
Residence **Germany**
Citizenship **Germany**
Post Office Address **Hilberstrasse 4**
D-12307 Berlin, GERMANY

Full name of fifth inventor, if any
Fifth inventor's signature   Date
Residence
Citizenship
Post Office Address

Full name of sixth inventor, if any
Sixth inventor's signature   Date
Residence
Citizenship
Post Office Address

Docket No.
SCH 1526

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROCESS AND COMPOUNDS FOR DETECTION OF ANALYTES USING REMANENCE MEASUREMENT, AND USE THEREOF

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 29 February 1996 as United States Application No. or PCT International Application Number PCT/EP96/00823
and was amended on ______________________
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Not Claimed
195 08 772.0	Germany	01 March 1995	☒
			☐
			☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112. I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

S1155

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

I. William Millen (Reg. No. 19,544)
John L. White (Reg. No. 17,746)
Anthony J. Zelano (Reg. No. 27,969)
Alan E.J. Branigan (Reg. No. 20,565)
John R. Moses (Reg. No. 24,983)
Harry B. Shubin (Reg. No. 32,004)
Brion P. Heaney (Reg. No. 32,542)
Richard J. Traverso (Reg. No. 30,595)
Diana Hamlet-King (Reg. No. 33,302)
John A. Sopp (Reg. No. 33,103)
Richard E. Kurtz (Reg. No. 33,936)
Richard M. Lebovitz (Reg. No. 37,067)
John H. Thomas (Reg. No. 33,460)
Luan Cao Do (Reg. No. 38,434)

Send Correspondence to: **MILLEN, WHITE, ZELANO & BRANIGAN, P.C.**
Arlington Courthouse Plaza I
2200 Clarendon Blvd., Suite 1400
Arlington, VA 22201

Direct Telephone Calls to: *(name and telephone number)*
Anthony J. ZELANO 703-812-5310

Full name of sole or first inventor **WEITSCHIES, Werner**	
Sole or first inventor's	Date
Residence **Germany**	
Citizenship **Germany**	
Post Office Address **Gneisenaustrasse 65**	
D-10961 Berlin, GERMANY	

Full name of second inventor, if any **KÖTITZ, Roman**	
Second inventor's signature R. Kötitz	Date 9.3.98
Residence **Germany**	
Citizenship **Germany**	
Post Office Address **Arnold-Zweig-Strasse** ~~12~~ 34	
D-13189 Berlin, GERMANY	

Full name of third inventor, if any **TRAHMS, Lutz**
Third inventor's signature Date
Residence **Germany**
Citizenship **Germany**
Post Office Address **Reinhardtstrasse 5**
D-12103 Berlin, GERMANY

Full name of fourth inventor, if any **BUNTE, Thomas**
Fourth inventor's signature Date
Residence **Germany**
Citizenship **Germany**
Post Office Address **Hilberstrasse 4**
D-12307 Berlin, GERMANY

Full name of fifth inventor, if any
Fifth inventor's signature Date
Residence
Citizenship
Post Office Address

Full name of sixth inventor, if any
Sixth inventor's signature Date
Residence
Citizenship
Post Office Address